RESPONSES OF NICOTIANA TABACUM CV. SR-1 TO XYLELLA FASTIDIOSA STRAINS

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ABSTRACT
Nicotiana tabacum genotype (SR-1), was evaluated as a susceptible host for the bioassay of Xylella fastidiosa strains. Readily transformable N. tabacum cv. SR-1 plants were propagated in vitro. Transplanted plants were inoculated with various Xf strains. Inocula consisted of aqueous suspensions of bacterial cells harvested from 7-10 day old cultures on solid PWG medium. Inoculations were made by needle puncture through 20μL of inoculum (10^8 bacteria/mL) placed in the axils of three basal leaves. Inoculated plants were maintained in a growth room (27-28°C, 12 hour photoperiod provided by GE High Output fluorescent lights) for 1 month, and subsequently transferred to a greenhouse. Generally, symptoms on plants inoculated with Xf strain Temecula-1 included necrosis at the margins with chlorotic zones extending toward the midvein after 6-8 weeks. Some affected leaves became cupped and curled downward. As infections became systemic, leaves that developed on new shoots were chlorotic and smaller. These symptoms did not develop on water-inoculated control plants. The presence of Xf in stems and leaf petioles of affected plants was confirmed by ELISA and real-time (RT) PCR. ELISA and RT-PCR assays of similar tissues from water-inoculated control plants were negative. Bacteria were observed by TEM and SEM in xylem cells in affected plants. No bacterial cells were observed in control plants. Xf was isolated from systemically infected tobacco leaf petioles from plants inoculated with Xf strain Temecula-1 and re-inoculated into grape plants cv. Ruby Seedless. Typical Pierce’s disease symptoms developed four weeks post-inoculation in the greenhouse, confirming the retention of pathogenicity of this strain to grapes after passage through N. tabacum cv. SR-1. N. tabacum cv. SR-1 plants with other Xf strains are being evaluated. Several factors, including plant age at the time of inoculation, method, and plant handling after inoculation, are being determined.

Twitching motility among various wild-type isolates and pilus-defective mutants of Xylella fastidiosa

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ABSTRACT
The genome of Xylella fastidiosa (Xf) contains at least thirty genes responsible for pilus assembly or function. Recently, it was shown that Xf possesses two distinct types of polar pili: long, type IV pili and short, type I pili. It was also demonstrated that the bacteria of the Temecula strain are able to move on a solid agar surface via type IV-pilus mediated twitching motility that results in the presence of a ‘fringe’ surrounding the expanding bacterial colony. Since our research had been limited to the Temecula strain, and since such colony morphologies had not been previously reported it was not known whether the fringe we observed in culture was an anomaly of the Temecula strain or if it was also a characteristic of other wild-type strains. We therefore examined fourteen isolates from California, Texas, and South Carolina. All but one Xf isolate developed a fringe around the colony periphery, suggesting that twitching motility may be a critical factor in the spread of the bacteria in planta and development of Pierce’s disease. We further discovered that fringe formation on PW agar is dramatically affected by the concentration of bovine serum albumin (BSA) in the medium. Type IV pilus-defective mutants, e.g., pilB did not develop a colony fringe. Mutants defective for the shorter type I pili, e.g., fimA continued to exhibit a fringe; and, in fact had a wider fringe.